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THE INFLUENCE OF TEMPERATURE ON THE RATE OF LOCOMOTION IN AMOEBA

I. LOCOMOTION AT DIVERSE CONSTANT TEMPERATURES[1]

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[1] Contributions from the Zoological Laboratory of the Johns Hopkins University. This and two papers that are to follow were submitted in 1921 in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Johns

INTRODUCTION

The present investigation was undertaken with the purpose of determining the quantitative relation of temperature to the rate of locomotion in Amoeba. In agreement with the results of other investigators, who have studied the influence of temperature upon the life processes of the protozoa, it was found that there is a correlation between temperature and the rate of locomotion. The statement occurs frequently in the literature bearing upon this point, that the rate of locomotion of other protozoa, as well as of Amoeba, increases with rising temperature. Davenport ('08) cites Rossbach ('72) and Schurmayer ('90) as having established different rates of locomotion of the ciliates, for different intervals on the temperature scale. These authors have pointed out that between 25° and 30° locomotion reaches an optimum for both rate and accurate coordination; that between 30° and 35° the high rate may be maintained, but the movement becomes very irregular; that, finally, near 40° progressive movements become slower and gradually cease. While these findings are definite enough in their delimitations of temperature conditions for locomotion of ciliates, evidently the reactions of the organisms are reported merely in descriptive and not in quantitative terms. For Amoeba, Rhumbler ('98) implies rather than states definitely the dependence of the rate of locomotion upon temperature. Similarly, Karl Gruber ('12) found that cooling retards the rate of locomotion of A. proteus, while an increase in temperature to 35° increases the rate. He states, however, that even at 30° this increase in rate is only temporary, and that a resting period ensues within a very short time. Schaeffer ('20) says of Amoeba: "In general, the lower the temperature the slower the movement. This has been frequently observed and recorded." Very little, therefore, seems to be known concerning the

Hopkins University. The problem was suggested by Prof. S. O. Mast, and the work was carried on under his direction. The author hereby acknowledges his great obligations to Professor Mast, to Prof. H. S. Jennings, and to his co-workers in the department.

exact quantitative measurement of the degree of dependence of locomotion and allied physiological processes on temperature in the protozoa, and specifically in Amoeba.

Regarding the absolute rates of locomotion in Amoeba, the data are very meager. The subject is treated for the most part in casual notes. Rhumbler ('98) tells us that A. verrucosa may move at a rate of 0.5 μ per second—at a rate, therefore, of 30 μ per minute. A. striata, according to the same author, moves at a rate of 60 μ per minute, as does also A. limax. Schaeffer ('20) estimates the rate of movement of A. proteus as 600 μ per minute, and in the present investigation the average rate of all the amoebae studied at various temperatures was found to be 625 μ—a result which is in very close agreement with Schaeffer's measurements. In discussing his own results, Rhumbler touches upon a feature of locomotor behavior, which will have to be stressed in the following pages, the variability of the rate of locomotion even at constant temperatures. One of his specimens of A. geminata, Pen., changed its rate of locomotion from 90 to 180 μ during five minutes, and he describes the occurrence of occasional rates of locomotion so rapid that they could not be estimated even approximately by the methods of measurement which he employed. It seems likely, as we shall see, that these variations in rate are manifestations of a locomotor rhythm.

Before proceeding, it seems necessary to define the term 'locomotion' as it is being used in this paper. Amoeba may pass from place to place by a variety of ways: *a*) It may be transported merely passively in a current of water. *b*) It may be transported passively while it exhibits protoplasmic flow. This Gruber ('12) has called 'activo-passive' transportation. *c*) It may progress actively by 'crawling,' that is, by protoplasmic flow, while it is attached entirely or by certain points of its subsurface to the substratum. This form of locomotion expresses a measurable physiological activity, and hence this investigation was concerned wholly with this method of progression.

During the last twenty years or more, it has become customary to express the dependence of a physiological process upon temperature in terms of a temperature coefficient. Thus far but few investigations have been undertaken with the special purpose of determining the temperature coefficient of physiological processes in the protozoa. Kanitz ('07) has calculated the temperature coefficient from the data of Rossbach ('72) and for observations of his own on the rate of pulsation of the vacuoles in several ciliates. Burian ('10, p. 263) calculated the temperature coefficient for the rate of pulsation of the vacuole in Vorticella, the data being taken from a paper of Ostermann's (54). Khainsky ('10) studied the same physiological process from this same viewpoint in Paramecium. The temperature coefficient of the rate of reproduction of the protozoa has also been studied in some forms. Blackman ('08) did this for Chilomonas from data in a paper of Maltaux and Massart ('06); Woodruff and Baitsell ('11 a) for Paramecium; Borowsky ('10) for Actinosphaerium. A third physiological phenomenon, the nucleocytoplasm relation in its dependence on temperature, has been studied by Jollos ('13) and by Rautmann ('09), but their data, while extremely valuable and interesting, are not easily expressible in terms of a temperature coefficient. In general it has been found that the temperature coefficient of physiological processes in the protozoa is of the same magnitude as that in higher forms. Thus far no study of the temperature coefficient of a locomotor process in the protozoa has been undertaken.

Our purpose in this investigation, then, was to study the influence of temperature upon the rate of locomotion in Amoeba, understanding locomotion to mean active locomotion.

A. Material

The amoeba used in this investigation were cultured from a strain found in Stony Run, a stream of spring water near the biological laboratories at Johns Hopkins University. This

stream is somewhat exposed to contamination from sewage and surface waters. The amoebae collected from the stream were cultured by the usual laboratory methods, consistent efforts being made to keep the various cultures as uniform as possible. The density of the population in the various dishes was carefully controlled and food conditions were kept optimal.

By far the greater number of individuals studied conformed to the diagnosis of Schaeffer's ('16) new species A. discoides. Some, however, conformed more nearly to Schaeffer's description of A. proteus. As these species were used indiscriminately, it is quite impossible at present to separate the experimental data with any degree of assurance.

In selecting a given individual for experimentation, its size and apparent activity were kept in mind. In the beginning of the work the amoebae were measured, but later the experience gained was deemed sufficient to insure fair constancy in the size of the individuals even without measurement. All the individuals selected were full grown and were between 300 μ and 450 μ in length during locomotion. Actually moving amoebae, of course, had to be chosen for the purpose of this work, but no long search was made to secure the most active in a given culture. It was felt that by such random selection individual differences would be most readily effaced in the averages.

More than one hundred amoebae were studied in this investigation, but the performance records of sixty-two only are used as the basis for this paper. These sixty-two amoebae were observed at one-minute intervals for a total of 5031 minutes, so that on an average each amoeba was under observation for eigthy-one minutes.

B. Methods

In its barest outline the investigation was conducted by measuring the rate of locomotion of amoebae under known conditions of temperature. To do this effectively and to facilitate the interpretation of the findings, it was necessary,

1) to secure a definite temperature in the immediate environment of the amoeba and to control this temperature; 2) to measure the temperature accurately; 3) to measure time; 4) to measure the distance traversed in a given unit of time; 5) to record the results and to represent them graphically.

1. Control of temperature. Water of known temperature circulating through the chamber of a Pfeiffer warming stage was used for controlling temperature conditions.

In the ordinary procedure, the animal to be studied is placed upon the upper surface of the Pfeiffer stage. It was found, however, that considerable time had to elapse before this surface became even approximately isothermic with the interior of the chamber, and that even after this had occurred

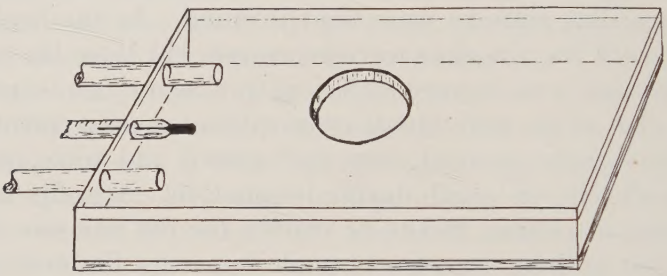

Fig. 1 The modified Pfeiffer warming stage.

the temperature fluctuated widely, owing to varying temperature conditions of the room and to the circulating air.

To obviate these difficulties, a circular opening, 2 cm. in diameter, was cut in the upper glass plate of the chamber (fig. 1). This opening was then closed with a glass disc, 0.28 mm. in thickness and 3 cm. in diameter, which was cemented to the inner surface of the plate with De Khotinsky cement. A cavity was thus formed in the upper plate of the Pfeiffer stage—hereafter to be spoken of as the 'depression cell.' The amoebae to be studied were placed into this cell in their own culture medium and were covered with a coverglass. They were thus separated from the water circulating in the chamber of the Pfeiffer stage by a sheet of glass only 0.28 mm. thick.

The water, delivered to the warming stage through glass and rubber tubing, came from one of a series of six 19-liter reservoirs, each of them kept at a definite temperature. These reservoirs were placed at a height of 50 cm. above the microscope stage and the connections were so arranged that water could be taken from any one of them. With this apparatus it was easy to effect a temperature change with any desired degree of accuracy and with great rapidity or to keep the temperature constant for any desired length of time.

2. Measurement of temperature. The temperature of the culture medium surrounding the amoebae under observation in the depression cell was measured by means of the electro-

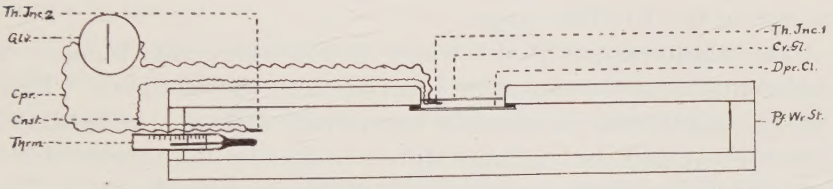

Fig. 2 Thermo-couple used with the Pfeiffer warming stage. *Pf.Wr.St.*, Pfeiffer warming stage; *Dpr.Cl.*, depression cell; *Cv.Gl.*, cover-glass; *Thrm.*, thermometer; *Glv.*, galvanometer; *Th.Jnc. 1*, *Th.Jnc. 2*, thermal junctions of couple; *Cpr.*, copper wire; *Cnst.*, constantan wire.

thermometer described by Hill ('13) and by Rogers and Lewes ('14), modified to meet the requirements of the present investigation.

The couples were formed of no. 41 constantan and no. 40 copper wire, a length of 50 cm. of the constantan wire being soldered at either end to pieces of copper wire, each 25 cm. long. The free ends of the copper wire were then soldered to leads of no. 28 copper wire, and these were connected to a galvanometer. The junctions were covered by dipping with a thin layer of paraffin. One of the junctions was attached by means of rubber tubing to the bulb of the thermometer and inserted into the chamber of the Pfeiffer stage; the other was placed loosely into the culture medium under the cover-glass in the depression cell (fig. 2). The galvanometer was

a D'Arsonval instrument (Leeds and Northrup, type P), mounted on an adjustable wall board, and had a resistance of 114 megohms and a period of 8 seconds. The scale was supported on the usual scale arm at a distance of 50 cm. from the mirror of the galvanometer. A straight scale was used, as it was intended to make readings at a short distance only on either side of the zero point.

The system was such as to give a deflection on the scale of the galvanometer of 9.5 mm. for a difference of one degree between the junctions. To ascertain the temperature of the culture medium surrounding the amoebae in the depression cell, it was necessary only to read the deflection on the scale of the galvanometer and to measure the temperature of the water in the Pfeiffer stage.

The temperature of the water in the stage was measured by means of a thermometer inserted into the chamber. This thermometer was repeatedly compared with a standardized instrument and its readings were checked by the temperature of both the inflowing and the outflowing currents.

The relation between the deflection on the scale of the galvanometer and the difference in temperature between the two junctions was ascertained empirically by taking the average of numerous readings on the scale with the junctions subjected to various known temperatures. In making the tests, one of the junctions was held in water of a given temperature and the other in water of a different temperature, the deflection on the scale being noted. The temperature was then changed in one or both of the vessels, and the deflection was again recorded. This was repeated until more than 500 records were obtained with temperatures varying from 9° to 34°. In these observations the deflection for one degree difference in temperature between the junctions varied from 9.3 to 9.8 mm. with an average of 9.5 mm.

Numerous observations were made on the relation between the temperature of the culture medium in the depression cell and that of the water in the Pfeiffer stage. Without going into details regarding the results obtained, the conclusions reached may be summarized as follows:

a. When the temperature of the water flowing through the Pfeiffer chamber was changed, the temperature in the depression cell responded immediately.

b. When the change from the old to the new temperature was not very great, the depression cell became isothermal, or very nearly so, within a time interval of less than one minute.

c. When the change from the old to the new temperature was rather great, and the new temperature was considerably different from room temperature, the temperature of the depression cell responded promptly, but still a difference of plus or minus 0.2° was maintained for rather a long time, perhaps for ten minutes—the sign of the maintained difference being dependent somewhat on the prevailing room temperature.

d. Even when the temperature which it was desired to maintain was considerably above or below room temperature, the cell sooner or later became practically isothermal with the chamber.

e. Once the cell had assumed the temperature of the chamber, or had approximated it closely, it maintained that temperature fairly constantly.

It should be explained that the value of our experimental data is not dependent upon the accuracy of these statements, for the reason that the temperature was directly and accurately ascertained for each observation. Greater accuracy could easily have been secured by this method, but it would have been beyond the experimental accuracy of other details of the investigation.

3. Measurement of time. In the early part of the work an Elgin watch was used. Later, however, a large Wizard alarm clock (Wizard Clock Company, New York) proved more serviceable. The second hand of this clock was found to be well synchronized with the minute hand, and preceded or lagged behind the minute hand by not more than five seconds during three hours.

4. Measurement of distance traversed. Using the microscope with the 16-mm. objective and the 6x ocular, camera-

lucida drawings were made of the posterior end of the moving amoeba at known time intervals, usually one minute. The distance between successive drawings was then measured and the rate per minute calculated. The drawings were all made upon sheets of paper of uniform size, 17.8 by 7.6 cm.

A series of drawings of the posterior end of a moving amoeba give an accurate picture of its path. In figure 3 are shown several of such paths, designated by capital letters, chosen almost at random from the records of this investigation. (The Roman numerals in the figure designate the individual amoebae as they appear in the records; the Arabic numerals are the numbers of the particular observations.) In some cases, e.g., in paths A and K, we find movement almost directly forward in the same direction. In others, e.g., B, C, D, the path is more or less curved. In others again, e.g., E, there is reversal of direction by a sharp turn in the path. In still others there may be complete reversal of direction by making the anterior end the posterior. Path H shows the condition of an amoeba resting along its path. In this case the animal was found in the same place when observations 3, 4, 5, and 6 were made. Path F shows a sudden turn in the forward movement, and path J, the difference one may meet with in the configuration of the posterior end during successive observations. Almost all of the paths here reproduced show considerable variation in rate, some of them of decided range. It is not to our purpose to enter here into a detailed discussion of this point.

For the purpose of measurement, the drawings in each path were connected by a line to facilitate measurement. A glance at figure 3 will show how this was done. In some cases there could be no doubt about the points that should be connected in establishing the path, e.g., paths A, B, G, H (fig. 3) are obvious. In other cases there was some question regarding the exact points through which the line should be drawn. Path K illustrates such a case. This path between observations 31 and 38 might have been made practically a straight line. Generally, however, such difficulties did not introduce an

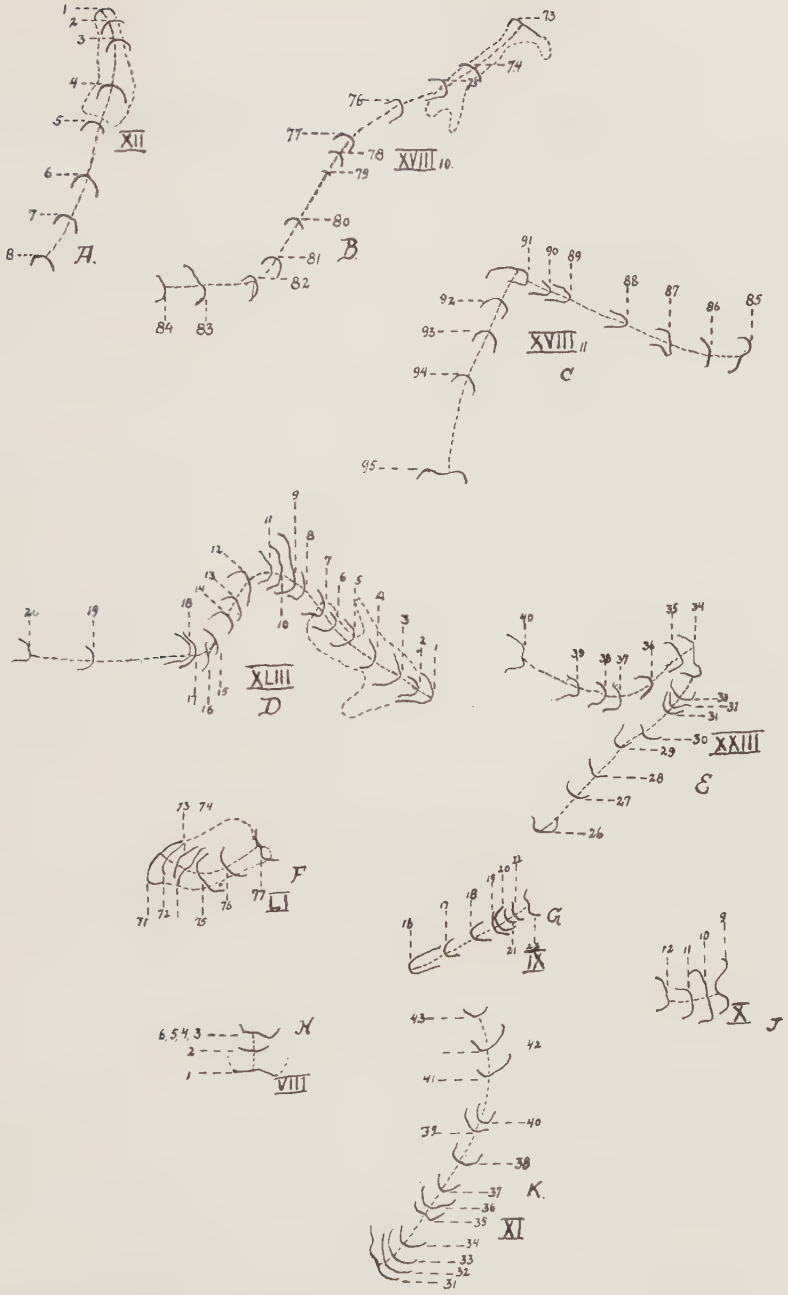

Fig. 3 'Paths' of Amoebae. Letters and Roman numbers designate individuals; Arabic numbers designate observations.

appreciable error into the measurements, as the difference in length between the various paths that might have been drawn and the one actually selected amounted to little more than two- or three-tenths of a millimeter.

The distances between the successive drawings were measured by laying a flexible steel tape graduated in millimeters upon the path, and reading off the intervals to the nearest half-millimeter.

Finally, the apparent rate was ascertained by dividing the distance traversed by the time interval elapsing between two successive drawings.

These values gave the 'apparent' rates as they were seen on the drawing-board. They were found to be magnified sixty-four times. They were not reduced to actual rates, however. In the following pages, therefore, when a given rate is mentioned, it must be understood that we are speaking of an apparent rate, and that the real rate may be found by dividing the apparent rate by 64. Thus, a rate of 5 mm. per minute is really an actual rate of 0.078 mm. per minute.

5. Tabulation and graphic representation of data. During the observations the following data were tabulated: 1) the time at which the observation was made; 2) the time interval since the previous observation; 3) the temperature of the Pfeiffer stage and the galvanometer deflection, from which two readings the actual temperature in the depression cell was calculated; 4) the distance traversed in the given time interval; 5) the average rate per minute during that time interval; 6) remarks on the details of manipulation.

From these data performance graphs were constructed for each individual amoeba. The variations in locomotor rate of each individual as well as the temperature conditions during the observations were both plotted against time. The behavior of the organisms during constantly maintained temperature conditions as well as at the instant when these conditions were changed could thus be seen at a glance.

6. Exclusion of other factors affecting the locomotor rate. It seems reasonable to suppose that the locomotor rate of

amoeba is controlled by many factors. Age, size, and nutritional conditions, as well as the physical density of the medium, its chemical constitution, light and temperature, all these and perhaps other factors as well undoubtedly affect the movement of this organism. An effort had to be made, accordingly, to equalize the influence of all factors except that of temperature. It was comparatively easy to do this with the external factors. Proper precautions are fairly effective in keeping the chemical constitution of the medium and its density uniform. The illumination, too, was kept definitely constant throughout the investigation, as all the observations were made in a dark room with the same electric bulb as the source of light.

Age could not be controlled satisfactorily. No definite statement can be made regarding the age of the different individuals used.

Size probably affects the absolute locomotor rate of an individual. It may be doubted, however, whether it affects the relative rate, i.e., the ratio of two rates at different temperatures. Considerable effort was made to secure individuals that were of comparable size as seen in optical section, but even this precaution is obviously no guarantee that size as a factor in the locomotor rate of different individuals has been equalized in all observations. It has already been stated that most of the amoebae studied were between 300 and 450 μ in length during locomotion.

That nutritional conditions were well equalized by being kept optimal is shown by the fact that so few individuals were found feeding, even though food was available in abundance.

OBSERVATIONS AND EXPERIMENTAL RESULTS

Prefatory remarks

1. The general conclusions. So great are the variations in the rate of locomotion of individual amoebae and so wide the divergencies in their behavior, that the following pages may easily convey the impression that a correlation between tem-

perature and the rate of locomotion is impossible of demonstration. For not only different individuals at varying temperatures, but even the same individuals at maintained temperatures, exhibit a most marked variability of rate. Yet, despite this fact, a study of the entire mass of data reveals a dependence of the locomotor rate on temperature. Hence, to forestall false impression, which may easily result from some of the statements that will be stressed below, it might be well to summarize here the chief general conclusions for which evidence will be presented.

1. The rate of locomotion increases with rising temperature from, approximately, 5°, the temperature at which no locomotion was observed, to a maximal value at, approximately, 22.5°. Beyond this temperature, the rate of locomotion decreases until it reaches zero at about 33°.

2. The dependence of the rate of locomotion on temperature is such that for averages of a large number of observations its measure can be expressed as a coefficient, in the van't Hoff sense, of between 5.4 and 1.89 for various temperature intervals throughout the entire range of temperatures used. This will be discussed more fully in a later paper.

2. Division of the subject. It would seem logical to substantiate these general conclusions under successive headings. For various reasons, however, this division of the subject has not been adopted. In the first place, it appeared desirable to describe at some length various phenomena associated with locomotion at constant temperature to which no, or only very casual, reference has been found in the literature. Changes of rate of locomotion under the influence of temperature can be understood only if the phenomena taking place under constantly maintained temperature conditions are known. Secondly, the reaction of amoeba at the instant of a change of temperature is markedly different from its 'normal' behavior in two constantly maintained temperatures. And, thirdly, the question of a possible measure of the dependence of rate of locomotion on temperature merits full discussion. Accordingly, the subject has been treated under the following headings, in separate papers:

Part I. Locomotion at diverse constant temperatures.
Part II. Locomotor response to changing temperature.
Part III. The measure of the dependence of the locomotor rate on temperature.

LOCOMOTION OF AMOEBA AT A CONSTANT TEMPERATURE

1. A typical performance record

A somewhat detailed description of the locomotor activity of an amoeba at constant temperature may prove helpful for the ready understanding of all that is to follow. We have selected individual XII for the purpose of this description not only because this individual was observed at minute intervals for the comparatively long period of two hours, during which the characteristic features of its locomotion could be ascertained, but also because during the observations on this particular animal certain difficulties were encountered, a description of which will probably not be without value for the further reading of this paper. The performance record (table 1) and the performance graph (fig. 4) for this individual are here inserted for the purpose of rendering the description more intelligible. It must be borne in mind that when we speak of rates in this section, we mean the rates as viewed under our optical system, that is, under a magnification of 64.

Individual XII, when placed in the depression cell of the Pfeiffer stage at 9.50 A.M., became attached to the bottom of the cell almost immediately and began to move promptly. The thermometer in the chamber of the stage at this time registered 19.5°, and this temperature was maintained throughout the two hours during which this series of observations was made. The galvanometer, however, indicated that the temperature in the depression cell fluctuated slightly between limits that were well within half a degree above and below the temperature of the chamber. As has been found from repeated experimentation, however, amoeba does not react in an appreciable way to such fluctuations when the prevailing temperature is very close to that of the environ-

TABLE 1

Performance record of individual XII (fig. 4)

NO. OF OBSERVATION	TIME	TIME INTERVAL	TEMPERATURE	TOTAL DISTANCE	RATE	REMARKS
		min.	*°C.*	*mm.*	*mm. per min.*	
1	9:55	0	19.5	0	0	
2	9:56	1	19.5	3	3	
3	9:57	1	19.5	4	4	
4	9:58	1	19.5	8	8	
5	9:59	1	19.5	9	9	
6	10:00	1	19.5	11	11	
7	10:01	1	19.5	9	9	
8	10:02	1	19.5	9	9	Animal under debris
9	10:16	0	19.5	0	0	New record sheet
10	10:17	1	19.5	7	7	
11	10:18	1	19.5	3	3	
12	10:19	1	19.5	3	3	
13	10:20	1	19.5	4	4	
14	10:21	1	19.5	8	8	
15	10:22	1	19.5	8	8	
16	10:23	1	19.5	11	11	
17	10:24	0	19.5	0	0	New record sheet
18	10:25	1	19.5	8	8	
19	10:26	1	19.5	7	7	
20	10:27	1	19.5	9	9	
21	10:28	1	19.5	4	4	
22	10:29	1	19.5	3	3	
23	10:30:05	1.08	19.5	8	7.4	
24	10:31	0.9	19.5	8	8.8	
25	10:32	1	19.5	4	4	
26	10:33	1	19.5	5	5	
27	10:36	0	19.5	0	0	Interruption New record sheet
28	10:37:05	1.08	19.5	4	3.7	
29	10:38:10	1.08	19.5	5	4.6	
30	10:39	0.83	19.5	7	8.4	
31	10:40:10	1.16	19.5	7	6	
32	10:41	0.83	19.5	7	8.4	
33	10:42	1	19.5	8	8	
34	10:43	1	19.5	10	10	
35	10:44	1	19.5	5.5	5.5	
36	10:45	1	19.5	9	9	Rest; began to move, 10:50

TABLE 1—*Continued*

NO. OF OBSERVATION	TIME	TIME INTERVAL	TEMPERATURE	TOTAL DISTANCE	RATE	REMARKS
		min.	*°C.*	*mm.*	*mm. per min.*	
38	10:50	0	19.5	0	0	New record sheet
39	10:51	1	19.5	5	5	Animal dragging debris
40	10:52	1	19.5	3.5	3.5	Animal dragging debris
41	10:53	1	19.5	3.5	3.5	Animal dragging debris
42	10:55	0	19.5	0	0	No observations made
43	10:56	1	19.5	3	3	Animal dragging debris
44	11:07	0	19.5	0	0	Animal under debris until 11:07
45	11:11	4	19.5	6.5	1.6	
47	11:16	5	19.5	7.5	1.5	Change of direction
48	11:21	5	19.5	9	1.8	
49	11:23	2	19.5	6	3	
50	11:25	2	19.5	7	3.5	
51	11:27	2	19.5	7.5	3.8	
52	11:29	2	19.5	10	5	
53	11:30	1	19.5	9	9	
54	11:31	1	19.5	5	5	
55	11:32	1	19.5	5	5	
56	11:32:30	0	19.5	0	0	New record sheet
57	11:33	0.5	19.5	5	10	
58	11:34	1	19.5	9.5	9.5	
59	11:35	1	19.5	6	6	
60	11:36	1	19.5	4.5	4.5	
61	11:38	2	19.5	5.5	2.8	
62	11:40	2	19.5	5	2.5	
63	11:42	2	19.5	10	5	
64	11:43	0	19.5	0	0	New record sheet
65	11:45	2	19.5	10	5	
66	11:47	2	19.5	9.5	4.8	
67	11:50	3	19.5	9	3	
68	11:52	2	19.5	15	7.5	
69	11:53	1	19.5	3	3	
70	11:55	2	19.5	6	3	
71	11:57	2	19.5	6	3	
72	11:59	2	19.5	6	3	
73	12:01	2	19.5	5	2.5	

ment in which the animal has been kept for some time. As individual XII had been kept in a culture at a room temperature of about 20°, we may safely assume that the slight fluctuations in temperature that were observed in the course of this experiment had little effect in determining the rate of locomotion.

An occasional glance at the performance graph will enable the reader to follow more closely the changes of rate which we are now about to describe. When the observations began,

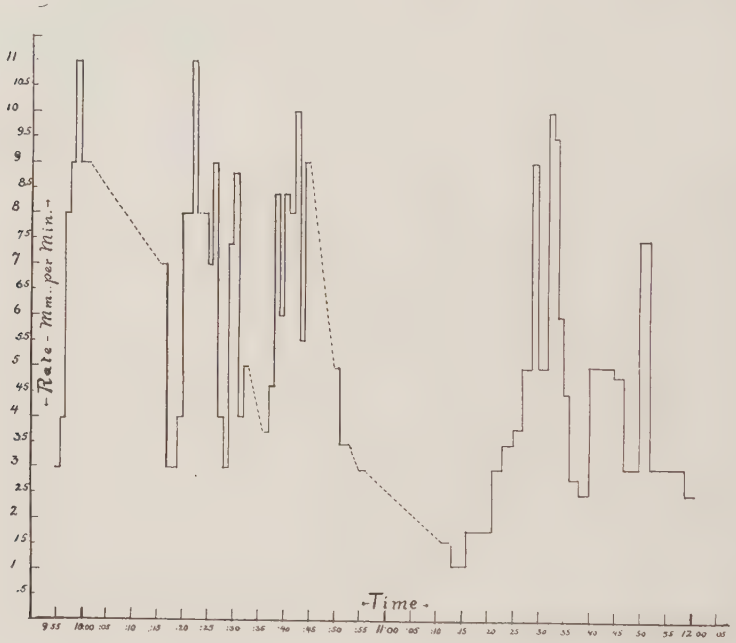

Fig. 4 Performance graph of Amoeba. Individual XII.

five minutes after the animal had been put upon the stage, the amoeba was moving at a rate of 3 mm. per minute. During the next four minutes this rate increased gradually to 4, 5, 8, and 9 mm. per minute, and during the fifth minute the rate reached a maximum value of 11 mm. per minute. The rate then decreased, the animal moving at a rate of 9 mm. per minute for the next two minutes.

The amoeba now encountered a mass of plant debris, crawled under it, and was lost to view from 10:02 to 10:16 A.M. When it emerged from the debris, its direction of movement was inclined at an angle of about 45° to its previous direction. As may be seen from the graph, there now followed, for seventeen minutes, from 10:16 to 10:33 A.M., a series of alternate accelerations and retardations of rate, some of them sudden, others more gradual. In one of these accelerations, the rate again reached its previous maximum of 11 mm. per minute, but this high maximum was followed by a gradual diminution in the magnitude of the accelerations. The minimum rate during this period did not fall below the previous minimum of 3 mm. per minute.

At 10:33 A.M., some manipulation of the reservoirs became necessary and the record sheet had to be changed, these operations consuming three minutes.

When the observations were resumed, the amoeba was moving at a rate of 3.7 mm. per minute. Another series of successive alternations and retardations now ensued, the rates being determined every minute for eight minutes. The maximum rate attained during this interval was one of 10 mm. per minute. A glance at the graph will, in fact, suggest a gradual slowing down of the rate. This phenomenon, as well as others which have been mentioned in this description, will be discussed below.

Between the rates of 6 and 10 mm. per minute (see graph), while the animal was moving at the rather high rate of 8.4 and 8 mm. per minute, the direction of movement changed through an angle of almost 90°, and a 'lunge' forward took place almost as soon as the animal had assumed the new direction.

During the next five minutes the amoeba was at rest. When it resumed its locomotor activity at a rate of 5 mm. per minute, it was dragging some plant debris, and its rate was slowed down to 3.5 mm. per minute for two minutes, after which it was again lost to sight for ten minutes under a mass of plant debris.

When it reemerged from the debris, the amoeba almost retraced its former path, and continued in the new direction along a path that was slightly sinuous, for the rest of the time during which it was under observation. At first its rate was rather slow, being only 1.6 mm. for four minutes, then 1.5 mm. for five minutes, and then 1.8 for the next five minutes. Then, however, a period of gradual accelerations began, as is clearly shown by the performance graph. The rate began to 'climb' at first to 3, then to 3.5, then to 3.8, then to 5, and finally to 9 mm. per minute. A retardation followed in which the animal was moving at a rate of 5 mm. per minute, and this in turn was followed by a rather sudden 'lunge,' during which a maximum rate of 10 mm. per minute was attained. The rate now decreased gradually, being successively, 9.5, 6, 4.5, 2.8, and 2.5 mm. per minute. The graph shows that this succession of rates must be interpreted as another 'wave' in the locomotion, a gradual increase to a maximum, and a corresponding decrease to a minimum of rate. Again, there was a rapid acceleration during which the amoeba sustained a rate of 5 mm. during five minutes, and this period of uniform motion was followed by a slight fall in rate to 4.8 mm. per minute and a greater one to 3 mm. per minute, but all this was preparatory to a 'spurt' during which a rate of 7.5 mm. was reached and sustained for two minutes. During the next seven minutes a uniform rate of 3 mm. per minute was kept, and when the series of observations was discontinued, the amoeba was moving at a rate of 2.5 mm. per minute.

This account of the locomotor activity of an amoeba during a period of somewhat more than two hours may be considered fairly typical of all the other individuals that have been studied. Sixty-two such performance records and graphs were made. These show that some of the amoebae rested for a longer time, while others were active for a longer time; that some attained much higher rates, others moved at lower rates; that changes of rate were more sudden in some cases, in others much less so. In general, however, a comparative study of the graphs emphasizes three features of locomotor

activity, which we are now to treat of at a greater length: A. Variability in the rate of locomotion at constant temperature. B. Various types of locomotion relative to change of rate. C. Rhythmic character of locomotion.

2. Some features of locomotion at constant temperature

A. Variability of rate. The description we have just given of the behavior of individual XII illustrates strikingly the first of the features of locomotion which must be stressed—the great variability of the rate. Temperature, in its relation to the rate of locomotion, cannot be thought of as determining absolutely the rate of progress. Temperature may fix a maximum rate which is not to be exceeded by an individual at that particular temperature, but below this rate amoeba may move at various rates. This becomes abundantly clear from even a casual inspection of the many performance graphs. This variability is exhibited not only, *a*) during periods of actual locomotion and, *b*) during periods of rest, but also, *c*) by an individuating characteristic proper to the various individuals, enabling the observer to designate each organism as 'slow' or 'fast.'

a. Variability during periods of actual locomotion. We have seen that individual XII did not exceed a rate of 11 mm. per minute at a temperature of 19.5°, but between that maximum rate and complete rest the animal assumed about twenty-five other rates, some sustained for a short period, others for a lengthy period. To choose another example at random, individual XLIV, in the twenty minutes during which it was kept at 18°, was moving at rates of 3, 7, 7, 7.5, 4.5, 3.5, 1.5, 2, 6.5, and 1 mm. per minute, in nine successive minutes. The same fact may be illustrated by a comparison of the rates of locomotion of different individuals, all at the same temperature. Table 2, excerpted from the records, enables us to make such a cursory comparison. The table shows the maximal, the minimal, and the average rates of locomotion of eight individuals all at 20°, in columns 3, 4, and 5, respectively. Column 1 gives the designation of the indi-

vidual. Column 2 is inserted to give some idea of the length of time during which such variations may occur, and in column 6 are given the number of different rates which were observed between the maximal and the minimal rate.

It will be seen from the table that in these eight individuals the maximal rates attained at 20° varied between 14.0 and 2.25 mm. per minute, the minimal rates, between 5.0 and 0.5 mm. per minute, and the average rates during the periods of observation, between 7.4 and 1.25 mm. per minute. In selecting them, no effort was made to present extreme examples.

TABLE 2

Different rates of locomotion of several amoebae at 20°

1	2	3	4	5	6
INDIVIDUAL NO.	DURATION OF OBSERVATION	MAXIMUM RATE PER MINUTE	MINIMUM RATE PER MINUTE	AVERAGE RATE PER MINUTE	NUMBER OF DIFFERENT RATES OBSERVED
	min.	*mm.*	*mm.*	*mm.*	
XIII	63	5.5	0.7	2.35	15
XIV	30	2.25	0.5	1.25	8
XVI	48	14.0	0.5	2.09	17
XX	84	8.5	0.8	2.46	16
XXX	31	13.0	1.5	6.33	16
XXXIII	30	5.0	1.5	3.46	6
XXXIV	33	14.0	5.0	7.40	11
XLIII	33	10.0	1.0	3.60	14

The conclusions seem justified that, 1) there is no fixed rate at which an individual must move at a given temperature; 2) at the same temperature, different individuals may move with decidedly different maximal, average, and minimal rates.

b. The periods of rest. Closely associated with the feature of locomotor behavior here being discussed is the length of the resting periods. The all but ceaseless locomotor activity of most of the amoebae that were studied is one of the most striking characteristics in the behavior of this organism under our experimental conditions. In some of the series of observations, an individual was followed for as long as four

hours, and in that length of time the resting periods all added together amounted to little more than twenty minutes. In some of these cases the animal was subjected to various temperatures, and it is possible that the stimulation imparted by this change of external condition shortened the periods of rest. But in other cases in which the amoeba was followed for two hours at constant temperature, the periods of rest were no less surprisingly short. The extent of variation of the length of the resting period, as well as its independence of temperature, may be illustrated by a few instances chosen at random.

TABLE 3

Duration of the periods of rest of several amoebae

INDIVIDUAL	DURATION OF OBSERVATION AT GIVEN TEMPERATURE	TEMPERATURE	DURATION OF LOCOMOTION	DURATION OF REST	REST PERIOD PER CENT OF DURATION DURING OBSERVATION
	min.	*°C.*	*min.*	*min.*	
L	45.5	18	42	3.5	7.7
XII	92.9	19.5	88.9	5	5.4
XX	80.5	20	52.5	28	34.8
XXII	57.5	25	55.5	2	3.6

Table 3 illustrates some of these points. From this table it will be seen that the four individual amoebae, nos. L, XII, XX, XXII, were observed for a sufficiently long time to give one a fair knowledge of their locomotor behavior, for 45.5, 92.9, 80.5, and 57.5 minutes, respectively. Moreover, these four individuals were observed at different temperatures, 18°, 19.5°, 20°, and 25°, respectively. In three cases the periods of rest were extremely brief, only 3.6, 5.4, and 7.7 per cent of the total duration of the observations, while in the fourth, the animal was resting during 34.8 per cent of the time during which it was under observation.

In general: 1) The resting periods in amoeba were found to be extremely brief. 2) At times an individual shows little locomotor activity during long periods of time. 3) Amoeba

may rest for a long or a short period at almost any temperature. 4) Amoeba is more likely to rest when the temperature is low than when it is high; unless the high temperature exceed the physiological optimum, when the periods of rest may be prolonged.

c. 'Fast' and 'slow' individuals. Despite all that we have said about the variation in the length of the periods of locomotor activity and of rest, it still remains true that different individuals manifest consistently a characteristic which might enable us to classify them as 'slow' or 'fast.' These adjectives characterize not merely their comparative rates, but also their periods of activity and inactivity in locomotion. It would be difficult to present in condensed

TABLE 4

'Fast' and 'slow' individuals, at 20°

INDIVIDUAL	RANGE OF RATES	AVERAGE RATE
	mm. per min.	*mm. per min.*
XIII	0.7– 6.5	2.36
XIV	0.5– 2.3	1.24
XXIV	2.0–16.0	9.5
XXXVI	4.0–14.0	7.6

form data which would substantiate this statement. Table 4 will emphasize the contrast. All the individuals listed in this table were observed at 20°. The designation of the individual is given in column 1, the range of rates at which the animal moved is given in column 2, and the average rate of locomotion during the period which the animal was under observation is given in column 3. Individual XIV, with an average rate of only 1.24 mm. per minute, might be characterized as 'slow,' which individual XXIV, with an average rate of 9.5 mm. per minute, is decidedly 'fast.'

The fact, however, that an animal moves slowly or rapidly at one temperature does not imply that it exhibits the same characteristic at another temperature. Table 5 illustrates the point.

In this table the rates of four individuals at 10° and 20° are compared. Individual XIV, which moved at a slow average rate of 1.24 mm. per minute at 20°, moved with a correspondingly slow rate of 0.60 mm. per minute at 10°. Individual VI maintained rates at these two temperatures which must be considered high. But individual XVI, which moved at a slow rate at 20°, moved at almost the same rate at 10°, and a rate of 2.00 mm. per minute must be considered high for this low temperature.

B. The change from rate to rate. A second feature of the locomotor activity of amoeba, which becomes apparent from a comparative study of the performance graphs, is the great

TABLE 5

'Fast' and 'slow' individuals, at 10° and 20°, compared

INDIVIDUAL	AVERAGE RATE	
	At 20°	At 10°
	mm. per min.	*mm. per min.*
XIV	1.24	0.60
XVI	2.40	2.00
V	8.20	1.50
VI	10.60	1.84

variation in the manner by which this organism changes from rate to rate. Amoeba may progress, *a*) at a uniform rate for an appreciable time; *b*) at a gradually accelerated or gradually retarded rate; *c*) at a suddenly accelerated or suddenly retarded rate; *d*) at a rate which is alternately accelerated and retarded.

a. Uniform rate. Only rarely is locomotion at a uniform rate maintained for more than two or three minutes. One individual (XII) maintained a uniform rate for seven minutes, another (XXVI) for four minutes. Methods of observation may be at fault in determining this point. It is obvious that if variations in rate occur with greater frequency than the observations, the variations will escape notice. Hence it becomes important to choose as small a time interval

as practical between successive observations. Again, sometimes when amoeba is seemingly at rest, it is in reality, progressing very slowly. This can readily be detected by using higher magnification. If the observer, therefore, waits before making his measurements until—under his optical system—he can determine the distance traversed during a given time interval, his readings may well give the impression that the individual has maintained a uniform rate for that given interval. Conclusions regarding the maintenance of a uniform rate can therefore be based only on such observations as are made at frequent and constant intervals of time. With such reservations the following statements may be made with some security:

1. Amoeba may move at a uniform rate of locomotion at any temperature within its range of tolerance.

2. Only rarely will the same rate of locomotion be found to have been sustained for two successive minutes. Sometimes such a uniform rate may be maintained for two or three minutes, and, in exceptional cases, for five and seven minutes.

3. Moderate or slow rates are more likely to be sustained uniformly for several minutes than are higher rates.

4. Amoeba is more likely to move at a uniform rate at lower than at higher temperature.

b. Gradually accelerated and gradually retarded rates. The observer frequently finds the rate of locomotion during successive minutes gradually increasing or decreasing. When such a series of observations is plotted, a 'staircase' graph will be obtained. The staircase may be upward if the acceleration is gradual, or downward, if the retardation is gradual, or it may be double, if both these phases are gradual. It is highly probable that in the animal itself the process represented by such graphs is a continuous one.

In figure 5 some of these 'staircase' graphs are shown. In the case of individual I (fig. 5, a) it took fifteen minutes to change from a rate of 0.2 to one of 4 mm. per minute with four intervening rates, and again during eight minutes its rate was retarded from 4 to 1.7 mm. per minute with three

intervening rates. The rate of individual XXXVIII (fig. 5, b) was changed during twelve minutes from one of 1.5 to one of 4 mm. with five intervening, gradually accelerating rates. Similarly, during the observations on individual XXVI (fig. 5, c) its rate was changed from 3 to 0.3 mm. per minute, with five intervening, gradually retarding during rates. The changing rate of individual XXXVIII (fig. 5, b), moreover, furnishes an excellent illustration of a double 'staircase.' Instances of this phenomenon might be multiplied, as they occur very frequently in many of the performance graphs.

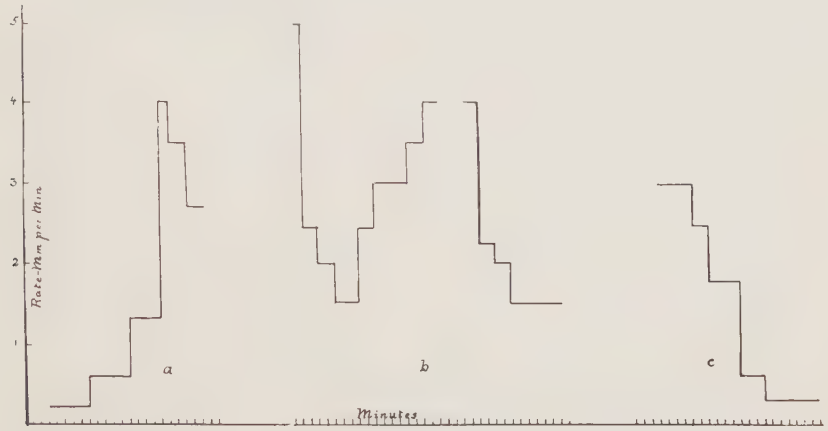

Fig. 5 Gradually accelerated and gradually retarded rates of locomotion. a, individual I at 9°; b, individual XXXVIII at 9.5°; c, individual XXVI at 19°.

This mode of behavior, moreover, is not characteristic of any particular temperature. The behavior just described was manifested by individual I at 9°; by individual XXXVIII at 9.5°; by individual XVII, at 15°; by individual XXVI, at 18°; by individual XI, at 23°. No clear cases of this phenomenon, however, are found at the higher temperatures, at those above 23°. Hence, 1) The rate of amoeba may be gradually accelerated or retarded, the change from one rate to another taking place very slowly. 2) This mode of changing the rate of locomotion may take place at any temperature, but it is much more frequent at lower and medium that at higher temperatures.

c. Suddenly accelerated and suddenly retarded rates. The change from one rate to another, however, is not always gradual. Very frequently amoeba may 'lunge' or 'jump,' sometimes from a very slow to a very rapid rate. A doubling of the rate of locomotion in two successive minutes is rather common, but some of the observations show that the rate of locomotion may increase even five-fold during successive minutes. The greatest sudden acceleration that was observed was found on the performance graph of individual XXXI—a change from 3.5 to 17.5 mm. in two successive minutes. Similarly, the retardations may be surprisingly sudden, the greatest observed being that of individual LIII—a change from a rate of 15.5 to 4.25 mm. per minute in two successive minutes. Observational difficulties in this case, too, must be guarded against, chiefly that of mistaking the passive or the active-passive transportation of the organism for active locomotion. We may conclude:

1. The rate of locomotion of amoeba may change very rapidly.

2. The rate of locomotion may increase five-fold or decrease by more than one-third in two successive minutes.

3. Such extreme changes are comparatively rare, however, while a doubled rate, or a rate reduced by one-half, occurs with comparative frequency.

4. Sudden accelerations are more common than sudden retardations, and the magnitude of a sudden acceleration is greater, generally, than that of a sudden retardation.

d. Alternately accelerated and retarded rates. By far more common than the modes of changing rates which we have just described is that of alternate accelerations and retardations. This mode of progression is well illustrated by individual XXVIII (fig. 6, a). In successive minutes the rates were 2, 3, 4, 3, 4, 2, 3, 4, 3, 4, 3.5, 5, 5, 6, 5, 5, 6, 5, 4, 5, 4, 7, 6 mm. per minute. The alternation of increasing and decreasing rates in the series is obvious. Individuals XIX (fig. 6, b), XXV (fig. 6, c), XLI (fig. 6, d), may be further cited as manifesting this feature of behavior rather strikingly.

This mode of behavior, like those previously described, also seems to be independent of a particular temperature. Still, it occurs more frequently at high than at low temperatures, though our data do not indicate that it occurs more frequently at high than at medium temperatures.

There are numerous detailed features of this alternation of accelerating and retarding phases, which it would be interesting to illustrate in detail from the individuals studied. It will have to suffice for the present, however, to make the following tentative statements, for which the graphs and

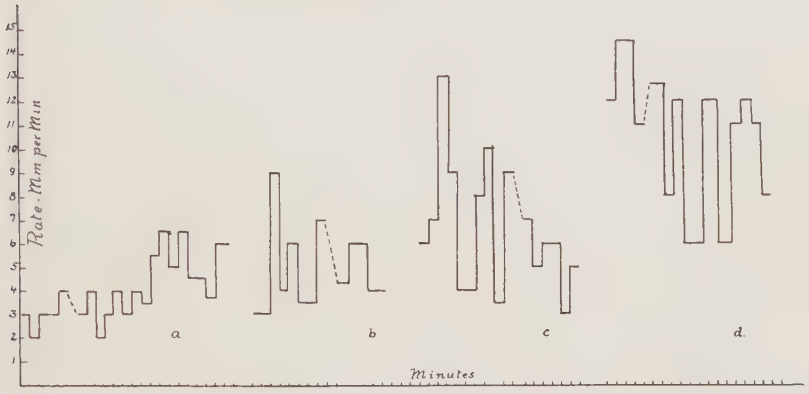

Fig. 6 Alternately accelerated and retarded rates of locomotion. a, individual XXVIII at 20°; b, individual XIX at 21°; c, individual XXV at 17°; d, individual XLI at 22°.

tables supply ample evidence. A more detailed study will be made below in the section dealing with the rhythm of locomotion. To summarize:

1. Amoeba may move with alternately increasing and decreasing rates.

2. Such a period of alternation may occur at any temperature, though it occurs more frequently at higher than at lower temperatures.

3. The duration of such a period of alternation is variable. It may be as brief as two or as prolonged as twenty minutes.

4. In periods of rather long duration, there may be occasional aberrations from the regular sequence, and an acceleration or a retardation of two or three minutes may occur in a series in which the alternations take place regularly every minute.

5. The duration of such a period of alternations is probably longer at higher than at lower temperatures.

6. Ordinarily, such a period occurs when amoeba is moving rather rapidly, though it may occur even when it is moving slowly.

7. In such a period of alternating accelerations and retardations, the extent of acceleration is apparently not dependent on the preceding retardation, nor is the extent of retardation dependent on the preceding acceleration, though, as will be seen below, some uniformity of behavior in this regard is detectable by careful measurement.

8. In a series, if the various accelerations show progressively increasing values, the intervening retardations may (frequently do) show decreasing values, though not necessarily so.

9. In a series, if the various accelerations show progressively decreasing values, the intervening retardations may show increasing values, though not necessarily so.

C. The rhythmic character of the locomotor rates. The mode of locomotion, by alternately accelerated and retarded rates, which we have just considered, leads us directly to discuss a third feature that is suggested by a study of the graphs—the rhythmic character of the changes in the locomotor rates. There are probably other forms of rhythmic activity which are associated with locomotion. Schaeffer ('20), for example, without speaking of rhythmic activity, has pointed out a dextrosinistrous, sinuous movement in the locomotion of A. bigema, as well as in other forms. Before him Calkins ('04), Mast ('10), and Woodruff in several papers have called attention to rhythmic activity in amoeba and in other protozoa. It is probable, moreover, that there is in amoeba a time-rhythm, which depends upon the relative

durations of the active and refractory periods, or, as they will be here called, the accelerating and retarding phases. Gibbs and Dellinger ('09, p. 241) say that "The amoeba proteus in common with higher animals has distinct periods of work and rest, depending for degree and duration upon the nature and abundance of food upon which the animal is habitually feeding." In all likelihood, various forms of periodic activity are, if not expressions of identically the same physiological states, still of closely related ones. Even from our own data upon this matter, it would seem highly probable that there is a close relation between the time-rhythm and the rate-rhythm, and suggestions are not wanting that both of these may be coincident with Schaeffer's directional rhythm. On the other hand, however, careful study has failed thus far to reveal the identity of Schaeffer's directional rhythm with the rate-rhythm we are here discussing, and the relation between the latter and the 'work and rest' rhythm of Gibbs and Dellinger seems even harder to establish.

The quantitative aspects of this locomotor rate-rhythm will merit extended discussion. Individual XXVIII furnishes a satisfactory instance, illustrating, as it does, not merely the remarkable coincidences which are common enough all throughout the performance records, but also the divergencies which still await more complete study.

a. An illustration. A part of the performance graph of individual XXVIII is reproduced in figure 7. The first observation made upon this individual showed that it was moving at a rate of 3 mm. per minute. The succeeding observations may be grouped in a series of periods, somewhat as follows:

Period 1	Retardation, 1 minute	Acceleration, 3 minutes
Period 2	Retardation, 1 minute	Acceleration, 1 minute
Period 3	Retardation, 1 minute	Acceleration, 2 minutes
Period 4	Retardation, 1 minute	Acceleration, 1 minute
Period 5	Retardation, 1 minute	Acceleration, 2 minutes
Period 6	Retardation, 1 minute	Acceleration, 1 minute
Period 7	Retardation, 3.5 minutes	Acceleration, 1.5 minutes

The periodicity here is unmistakable. There are alternate accelerations and retardations. In addition to this, however, the alternate accelerations, those, namely, of periods 1, 3, 5, 7, are slower than those of the intervening accelerations, those of periods 2, 4, 6. In the effort to find the underlying sequence of these coincidences, a 'ratio of rates' was used. We shall now have to explain this in some detail.

b. The ratio of rates. 1. The ratio of rates for one individual. During the retardation of the first period, the animal

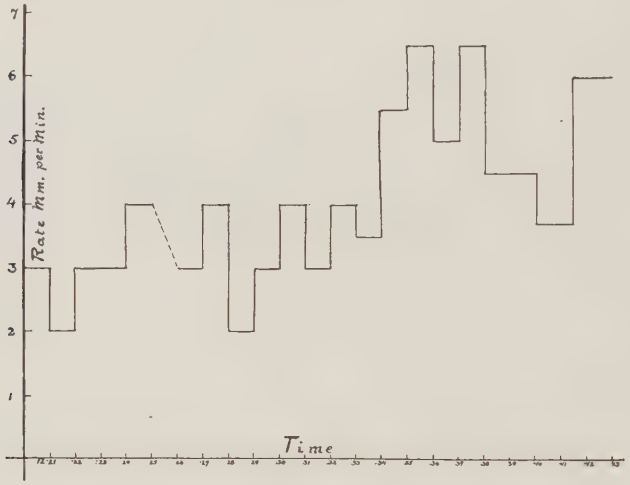

Fig. 7 Part of the performance graph of individual XXVIII, to illustrate the rhythmic changes of rate.

was moving at a rate of 2 mm. per minute. During the acceleration of this period, the animal moved at a rate of 3 mm. per minute for two minutes, and at a rate of 4 mm. for one minute. Hence, during this accelerating phase, which lasted three minutes, the amoeba moved a total distance of 10 mm. and, therefore, at a rate of 3.33 mm. per minute. If we now divide the rate maintained during the accelerating phase by that maintained during the retarding phase, the quotient will give us a measure of the number of times by which the accelerating rate exceeded the retarding rate. We

may call this value the 'ratio of rates,' and its mathematical value will be indicated by the expression R_{ac}/R_{rt} throughout the present discussion. During the first period of the locomotion of individual XXVIII, which we have been considering, the value of this ratio is 3.33 mm. divided by 2 mm., or 1.66.

TABLE 6

Rhythm in the locomotion of individual XXVIII during twenty minutes (graph, fig. 7)

PERIOD	RETARDING PHASE			ACCELERATING PHASE			R_{ac}/R_{rt}
	D	T	R_{rt}	D	T	R_{ac}	
	mm.	*min.*	*mm. per min.*	*mm.*	*min.*	*mm. per min.*	
1	2	1	2	6	2		
				4	1		
				10	3	3.33	1.66
2	3	1	3	4	1	4	1.33
3	2	1	2	3	1		
				4	1		
				7	2	3.5	1.75
4	3	1	3	4	1	4	1.33
5	3.5	1	3.5	5.5	1		
				6.5	1		
				12.0	2	6	1.71
6	5	1	5	6.5	1	6.5	1.30
7	9.0	2					
	5.5	1.5					
	14.5	3.5	4.1	9	1.5	6	1.46

D, distance traversed by animal during stated period; T, time interval; R_{ac}, rate during acceleration; R_{rt}, rate during retardation.

For the second period the value of this ratio is 1.33; for the third, 1.75; for the fourth, 1.35; for the fifth, 1.71; for the sixth, 1.30, and for the seventh, 1.46. Table 6 summarizes these facts, and shows, moreover, how these values were derived.

From this table, especially if it be studied with the graph, figure 7, the following points will become clear:

a. There was a very evident rhythm in the locomotion of this individual.

b. This rhythm expressed itself not merely in an alternation of accelerations and retardations, but also in an alternation of periods of greater with those of less acceleration (compare the ratio of rates for periods 1, 3, 5, 7, on the one hand, with those of periods 3, 4, 6, on the other).

c. The value of the ratio R_{ac}/R_{rt} was noticeably constant in alternate periods.

TABLE 7

The value of R_{ac}/R_{rt} for some amoebae chosen at random

INDIVIDUAL	PERIOD	R_{ac}/R_{rt}
XII	1	1.2
	2	2.6
	3	2.0
XIX	1	2.25
	2	1.70
	3	1.60
	4	1.50
XXV	1	1.52
	2	2.60
	3	1.50
	4	2.00
XLI	1	1.27
	2	1.60
	3	2.00
	4	1.20
	5	1.20

Other instances of such striking regularity in the rhythmic locomotor rate might be given. Without laboring the point, however, it may be said that the tendency to maintain such a rhythm is obvious throughout the data, although the rigorously quantitative character of such a rhythm is subject to wide variations. It is possible that observational difficulties again may account for much. To show the general character of these variations, table 7 has been compiled, the instances being chosen at random.

It will be noted that individual XXV shows the same alternation of greater and smaller values for the ratio which we have discussed for individual XXVIII, the values for the first and third periods being practically equal and small, those for the second and fourth periods being comparatively large. For individual XII the data are insufficient to enable one to discuss the quantitative character of the rhythm. Individual XIX moved in such a way as to gradually reduce the value of the ratio of rates. Individual XLI, on the other hand, moved in such a way as to increase the value of the ratio of rates, and then to make the value of this ratio constant. Such cases will be discussed at greater length when we speak of the long-time rhythm of locomotion.

2. The ratio of rates for several individuals at the same temperature. In discussing the rhythm of the locomotor rate in amoeba, we have considered, thus far, the ratio of rates for the successive accelerations and retardations in the locomotion of one individual. Another rather striking feature of this rhythm is the comparative constancy of the value of this ratio, for the average rates during accelerating or retarding phases for different individuals at the same temperature. If we add the distance traversed by a given individual during all the periods of acceleration observed at a given temperature, and divide this distance by the total duration of the accelerating phases, we shall get the average rate maintained by the animal during the accelerating phases. Similarly, the average rate maintained during the retarding phases may be found. If we now get the ratio of rates, by dividing the average rate during the accelerating phases by the average rate during retarding phases, we shall find by how many times the average accelerating rate exceeded the average retarding rate.

Table 8 will illustrate the treatment of our data for the present purpose in the case of individual XXIV. The table is divided vertically into two halves, one for the accelerating phases, the other for the retarding phases. In the first column in each half is given the distance traversed by the animal

during a given time interval, in one case during the accelerating, in the other during the retarding phase; in the second column is given the time interval during which that distance was traversed.

TABLE 8

The ratio of average rates of the locomotor rate rhythm. Individual XXIV at 20°

PERIOD	ACCELERATING PHASE		RETARDING PHASE	
	Distance	Time interval	Distance	Time interval
	mm.	*min.*	*mm.*	*min.*
1	22	2	8	1
			5	1
2	14	1	8	1
			12	1.5
3	8	1	14	2
	14	1	3	1
			2	1
4			13	1
			10	1
			8	1
			7	1
	22	2	6	1
5	12	1	8	1
	14	1	6	1
6			9	1
	16	1	8	1
7			15	1
	13	1	12	1
	135	11	154	19.5

Average rate during accelerating phase, $R_{ac} = 12.3$ mm.

Average rate during retarding phase $R_{rt} = 8.0$.

$$\frac{R_{ac}}{R_{rt}} = \frac{12.3}{8.0} = 1.5$$

If, now, we add all the values given in the distance column under 'accelerating phase' we shall get the total distance traversed by the animal during the duration of all the accelerating phases of its rhythm. If this value is divided by the

sum of the time intervals, we shall get the average rate of this individual during all its accelerating phases. The total distance traversed by individual XXIV was actually 135 mm. during 11 minutes, and, therefore, its average rate during the accelerating phases was 12.3 mm. per minute. Treating the data under the 'retarding phase' in the same way, we

TABLE 9

The ratio of average rates of the locomotor rhythm of amoebae at 20°

INDIVIDUAL	ACCELERATING PHASE			RETARDING PHASE			R_{ac}/R_{rt}
	D	T	R_{ac}	D	T	R_{rt}	
	mm.	*min.*	*mm. per min.*	*mm.*	*min.*	*mm. per min.*	
XIII	90.0	26.0	3.45	57.1	31.0	1.84	1.9
XVI	50.5	16.0	3.10	47.0	26.5	1.80	1.7
XVII	122.6	15.5	7.90	107.5	19.5	5.50	1.4
XVIII	127.5	33.5	3.80	52.0	34.0	1.50	2.5
XVIII	58.0	13.5	3.60	40.0	17.5	2.30	1.6
XIX	96.0	22.5	4.30	41.5	17.5	2.40	1.8
XX	94.3	27.5	3.40	36.2	24.5	1.50	2.3
XXIII	94.0	15.5	6.10	54.0	13.0	4.10	1.5
XXIV	135.0	11.0	12.30	154.0	19.0	8.00	1.5
XXX	65.5	8.0	8.20	82.0	16.0	5.10	1.6
XXXI	56.0	11.0	5.10	45.5	17.0	2.70	1.9
XXXIII	75.5	17.5	4.30	21.0	12.0	4.20	1.0
XXXVI	112.0	13.0	8.60	120.0	17.0	7.00	1.2
XL	66.5	9.0	7.40	65.0	12.0	5.40	1.4
XLIII	46.5	9.0	5.20	40.0	15.0	2.70	1.9
XLIII	68.0	13.5	5.00	29.0	8.0	3.60	1.4
	1376.35	276.0	5.18	1050.3	317.5	3.40	1.5

D, distance traversed by animal during stated period; T, time interval; R_{ac}, rate during acceleration; R_{rt}, rate during retardation.

find that the average rate during the retarding phase was 8 mm. per minute. Hence, the value of R_{ac}/R_{rt} in this case is 12.3 mm. divided by 8 mm. which gives 1.5 as the value of our ratio of average rates. This means that on the average the animal during the accelerating phases moved 1.5 times as fast as it did during the retarding phases.

Now, if we subject the data for a number of individuals, all of which were observed at 20°, to the same sort of analysis, it will become apparent that the value for the ratio of average rates is strikingly constant. The results of such a study are embodied in table 9. The table is constructed very much like table 6, for which the explanatory details have been given. It will be noted that even though the value of the accelerating rate (R_{ac}) varies between such wide limits as 3.10 mm. per minute for individual XVI and 12.3 mm. per minute for individual XXIV, and the value of the retarding rate varies between 1.8 mm. per minute and 8 mm. per minute for the same two individuals, respectively, the ratio of average rates for all of these individuals lies between 1 and 2.5. The value of the ratio of average rates for all of these individuals is again 1.5.

The only basis for the selection of the individuals for which the results were to be included in this table was the length of time during which they were observed. Those only were chosen which had been observed in a locomotor condition at 20° for twenty minutes or more, so that there might be some assurance that the characteristic features of the activity had actually been found.

3. The ratio of rates at different temperatures. Finally, if we compare the value of the ratio of rates for several individuals at different temperatures, another feature will become apparent. Unfortunately, however, our data are not adequate for forming a definite conclusion on this matter. Still, an indication of a possible trend might prove suggestive. If we select a number of individuals at different temperatures, choosing only those that have been observed for a fairly long time, and then treat their records as the data for individuals at 20° were treated, we shall notice a gradual diminution of the value of the ratio of rates as the temperature rises. The evidence is presented summarily in table 10. To show how much value is to be attached to the results for the various temperatures, the number of individuals from the records of whose performance the data are derived, is given in the

first column. The second column gives the temperatures at which the observations were made. The remaining part of the table is compiled as was described previously for table 6.

It is to be regretted that the data for some of the temperatures are so meager. It is thought probable, however, that the general trend of the data would not be changed even if the experimental findings were more numerous. No effort was hitherto made to work over the data for the intermediate temperatures.

It will be noted that at 15° the value of the ratio of rates is 1.8; at 18° it is 1.57; at 20° it is 1.5, and at 25° it is 1.28.

TABLE 10

The ratio of average rates of the locomotor rate rhythm of amoebae at various temperatures

NO. OF INDIV. OBSERVED AT GIVEN TEMP.	TEMP.	ACCELERATING PHASE			RETARDING PHASE			R_{ac}/R_{rt}
		D	T	R_{ac}	D	T	R_{rt}	
	°C	*mm.*	*min.*	*mm. per min.*	*mm.*	*min.*	*mm. per min.*	
4	15	178	56.75	3.13	172.5	94.75	1.72	1.8
5	18	638.5	83.00	7.70	424.5	87.50	4.90	1.57
16	20	1376.35	276.00	5.18	1050.3	317.50	3.40	1.50
4	25	652.0	64.58	10.10	576.5	73.00	7.90	1.28

D, distance traversed by animal during stated period; T, time interval; R_{ac}, rate during acceleration; R_{rt}, rate during retardation.

The progressive downward value is quite evident. We shall come back to a discussion of this phenomenon after we have treated the following section—the long-time-period rhythm in the locomotion of amoeba.

c. The long-period rhythm. In addition to the periodicity manifested by an alternation of accelerations and retardations, there became evident in some of the amoebae used in this study another long-period periodicity, the full meaning of which will need further investigation. This long-period rhythm, as we shall hereafter designate it, can become manifest only when one individual is studied at the

same temperature for a prolonged time interval. In this matter, as in many others connected with amoeboid movement, it is most important to note Schaeffer's warning. He says ('20, p. 109): "It has been tacitly assumed that if one could explain amoeboid movement at any particular cross-section in time, one understood the whole process of amoeboid movement no matter how long it continued. . . . It was not assumed that time was an element in the practical sense in the explanation of locomotion." Individual XVIII will serve as an illustration. To facilitate description, the performance graph for this individual has been redrawn on an enlarged scale, some of the smaller fluctuations of the rate have been omitted, and the rates for every fifth minute only (in one case for a four-minute interval) have been plotted (fig. 8).

The alternations of accelerating and retarding rates become evident at once. But just as evident is the gradual decrease of the accelerating rates from 12:05 to 1:00, its gradual increase to 1:25, then again its decrease until 1:45 o'clock, its increase to 2:05 o'clock, and finally, probably, its decrease beyond this time. It is clear that a very long time period of continued observation of the same individual at constant temperature is required to reveal so complex a phenomenon.

As our data for the understanding of this long-period rhythm are so meager, little more can be said about it. Still certain features which we have alluded to above may be mentioned in passing, as it is possible that further knowledge of the long-period rhythm may help us in understanding some of the features of the short-period rhythm.

Granting that such locomotor rate long-period rhythms do occur, it will be obvious, first of all, that the performance graph of an amoeba, even for a comparatively short time interval, will look differently, depending on whether it represents the condition of the animal in the downward or the upward phase of the long-period rhythm. This may explain the decided difference in the appearance of the graphs of different individuals at the same temperature. To further illustrate the point, we might turn back to the performance

graph of individual XII (fig. 4). In this case there is at first an upward tendency of the line connecting the various maxima, then a downward tendency, and again an upward tendency. This would seem to mean that a wave and a half of the cycle was studied: In the case of individuals XIX and XVI, there is clearly a downward tendency of the line connecting the maxima. The graph of individual XVII, on the other hand, seems to indicate that locomotion of this amoeba was studied while the animal was at the crest of a long-period rhythm. Instances of this phenomenon might readily be multiplied.

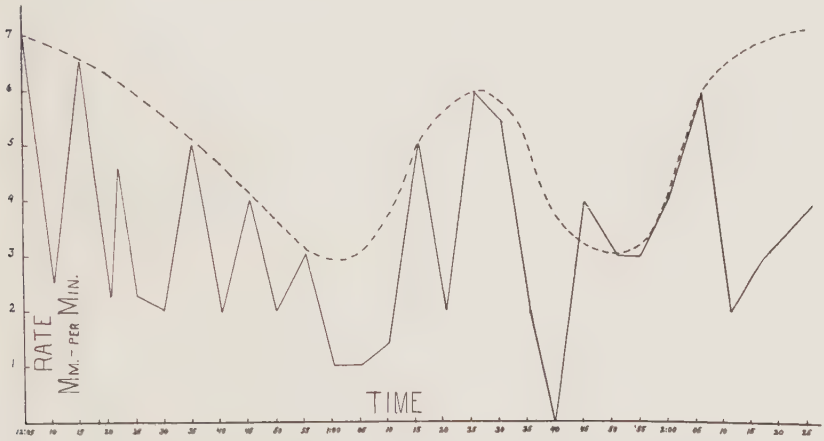

Fig. 8 Long-period and short-period rate rhythms during locomotion. Individual XVIII. ————, short-period rhythm; – – – – –, long-period rhythm.

Thus far we have spoken of the curve connecting the maxima of the rates of locomotion. Other interesting relationships would probably be revealed, if our data were ample enough to warrant an extensive discussion of the line connecting the minima. A few considerations, by way of suggestion rather than of definite statement, may not be out of place. If the curve of minima runs parallel to the curve of maxima, the curves must be interpreted as meaning that for every increase or decrease in the accelerations, there is a corresponding increase or decrease in the retardations.

Under such conditions, clearly we should expect the ratio of rates (defined above) to remain constant. Again, if the curve of minima converges toward the curve of maxima, the meaning is probably this, that while the acceleration is increasing or decreasing, the retardation is varying in an opposite sense. In such a case, we should expect the ratio of rates gradually to decrease in value. Thirdly, if the curve of minima diverges from the curve of maxima, we should expect the ratio of rates gradually to increase.

Lastly, since in all these three cases the curve of minima is certain to run parallel to the curve of maxima for some time, near the central region of each 'wave,' we should expect to find in the performance graph of each individual a number of alternate accelerations and retardations for which the ratio of rates is fairly constant. All these various hypotheses might readily be illustrated from the data accumulated during this investigation, and the number of verifications of such suppositions are sufficiently numerous to warrant this brief mention.

3. A discussion of certain features of locomotion of amoeba at constant temperature

We may now summarize what has been said about the locomotion of amoeba at a constant temperature. It is not our purpose in this place to study the bearing of our data on a theory of the mechanics of amoeboid movement. Our purpose is rather to suggest a relation which may possibly exist between the features of locomotion which we have been describing and the physiological condition of the amoeba during its locomotor activity.

1. Locomotion in amoeba is rhythmical.

2. The rhythm manifests itself in several ways during locomotor activity, and one of these ways is a periodic variation in the rate of locomotion.

3. This locomotor-rate rhythm is probably two-fold, *a*) a rhythm of short duration, consisting of alternations of accelerations and retardations and, *b*) a rhythm of long dura-

tion superimposed upon the short periods and apparently governing the magnitude of the accelerations and retardations.

4. In both rhythms the accelerating and retarding phases are variable, *a*) in intensity—the amount of change effected in the velocity; *b*) in duration—the length of time during which the changes persist.

5. In the short-period rhythm the relation between the two phases is expressible by a ratio, the value of which, depending on the relation between the curves of maximal and minimal rates, may be constant or may show an increase or a decrease. The magnitude of the ratio, however, varies with the temperature, decreasing with rising and increasing with falling temperature.

6. At any given instant of time the rhythm may be obscured or interrupted, for the rate of locomotion—presumably the rhythm—may change in a great variety of ways, thus for a time, giving the appearance of 'random variation' in rates.

7. During a prolonged period of active locomotion, however, the periodicity of rates becomes obvious even after very great and frequent interruptions.

These details of the locomotor rhythm of amoeba are of so striking a character that they invite an effort at interpretation. Without committing ourselves to any one of the numerous theories regarding amoeboid movement, it is rather our intention to outline in some detail those features of locomotor behavior which this investigation has either revealed or specially stressed. These will have to be considered in any adequate theory of amoeboid movement.

Every student of amoeba is familiar with the 'eruptions' of granules, which, occurring in the endosarc, seem to arise from a 'dynamic center,' and either initiate locomotion or are themselves the expression of some other process which initiates it. These eruptions take place at more or less irregular intervals of time, and are followed by longer or shorter periods of gradually retarding flow. From extensive observations it can be stated with fair assurance that the

variations in the rate of locomotion parallel the series of eruptions, and that the accelerated phases of locomotion occur synchronously with the eruptions. The eruptions, accordingly, release accumulated energy. This released energy, in turn, is utilized or dissipated during the 'refractory' or the 'dissipative' phase, while at the same time, the storage of materials, or some other similar process is going on in the 'dynamic center' of the organism, preparatory to the next eruption. In other words, the rate-rhythm may be conceived, as so many other rhythmical, biological processes have been conceived, as the expression of a reversible metabolism of energy. In the eruptive phase potential energy is converted into kinetic energy; in the refractory phase potential energy is being 'accumulated' preparatory to the next period of release.

The eruptions may vary, 1) in frequency, 2) in intensity. The frequency of the eruptions conditions the 'closeness' of the rhythm, the number of rhythmic waves that are packed into a given time interval. Now, this frequency can be altered in only one fundamental way—by a change in the time interval between the various eruptions. But the relation in duration between the eruptive and the refractory phases may be altered in a great many ways: *a*) The eruptive phase may be long and the dissipative phase short; *b*) the eruptive phase may be short and the dissipative phase long; *c*) an eruption may occur at the end of the dissipative phase of the previous eruption or after that dissipative phase has barely begun, or at any intervening point of time between these two moments.

The eruptions may vary, moreover, in intensity. The intensity of the eruption will determine, at the instant when it occurs, the magnitude of the acceleration of rate.

The dissipative period, too, may vary, at least, in duration. The observational basis for this statement is the fact that the rate of locomotion may be retarded suddenly or gradually. Since two processes, as we have said, are taking place in the organism during this period, each of these again may have

its own velocity. The ultimate resultant of both these processes, however, will finally be expressed in the magnitude of the succeeding retardation.

All of this might well be developed at much greater length. Since the variations in rate are plotted as 'waves' on the performance graphs, the whole subject of wave motion might supply endless suggestive analogies. Actual instances of the rate of locomotion of amoeba might, without laboring the point, be interpreted in terms of interference phenomena in the waves of the rhythm. The qualitative and quantitative variations of the locomotor rates of Amoeba may accordingly be described in terms of the rhythmically operative factors with their variations in frequency and intensity. Still the locomotor behavior must not be conceived as a comparatively simple manifestation of physiological conditions. The interplay of the environment, on the one hand, and of the physiological factors, on the other, must necessarily be of so complex a character that it is definable by no simple formula.

It remains to point out the possible applications of all this to some of the outstanding modes of locomotor behavior which we have described.

1. We have seen that amoeba may move at a uniform rate for rather a long time interval. We may well interpret this as due to a series of very frequent eruptions of small intensity—so small, that to be detected, their resultant accelerations would have to be measured under a higher magnification and at shorter time intervals than was done in this investigation.

2. Amoeba may move at a suddenly greatly accelerated rate. The acceleration, in this case, is probably due to a sudden, almost instantaneous eruption of great magnitude.

3. Amoeba may move at a gradually accelerated rate. Such an acceleration is probably due to a slow eruption of greater or smaller magnitude. In this form of motion, as we have seen, the eruption may be so slow that several different measurable rates may be found in as many as six successive minutes, during which the rate of the eruption con-

stantly increases, giving what we designated as a staircase mode of change of rate.

4. Amoeba may move at suddenly or gradually retarded rates, these again varying in their magnitude. Such retardations may be interpreted as due to varying durations of the refractory periods and to different intensities of the processes that take place during that period.

5. The constancy of the ratio of rates at a given temperature will also probably be found to be explainable on the basis of rhythm.

Before proceeding with the discussion of this statement, a note must be inserted. In this discussion of the rhythm, of the rate of locomotion, and later, in the discussion of the possible meaning of variations in the value of the temperature coefficient, we shall take occasion to refer repeatedly to Woodruff's ('11, 11 a, '17) papers on the reproductive rhythm in Paramecium. The comparison between our short-period and long-period rhythm, on the one hand, and Woodruff's 'rhythm and cycle,' on the other, would seem to demand some vindication. As was pointed out before, other workers in the protozoa have found rhythms in the reproductive activity and in other physiological processes of the lower forms. But as Woodruff has treated this matter professedly, his results will most readily supply suggestive analogies of the phenomena now being discussed. Woodruff's 'rhythm and cycle' were of much longer duration than the short-period and long-period rhythms of which we are speaking. In our comparison, therefore, we are merely comparing certain features of rhythmic activity, which Woodruff has pointed out, with those that seem discoverable in the present work, and are in no way implying a belief in the identity or even comparability of the physiological processes involved.

Now, Woodruff ('11 a), in the paper in which he established the existence of the rhythm in the reproductive activity of Paramecium, says: "It should also be pointed out that the total number of divisions during a prolonged period of time is comparatively constant. For example, the number of genera-

tions attained by a culture during 1909 was 613, and during 1910 was 612. Of course, this very exact coincidence is an 'accident' but taken with a considerable amount of data along the same line, it quite definitely points to the fact that the organism has the potential for about a certain number of bipartitions during a long period of time and this number is approximately attained irrespective of the minor fluctuations in the rate, due to external or internal causes.''

If this may be said concerning a process like reproductions, which, presumably, is so much more complicated than the rhythm of the rate of locomotion which we are here discussing, the constancy of the ratio of rates does not seem so very surprising. We may, therefore, conclude with some degree of probability that—to parallel Woodruff's statement—amoeba has a certain potential for a given amount of locomotion during a long period of time, and this amount is practically constant—unless, as happens in our case, external factors, temperature, for instance, so influence that potency as to increase or decrease it. What the concrete, objective meaning of this potency is, cannot be said with definiteness. That it is associated in the present case with certain variations in the elasticity and fluidity of protoplasm under the influence, direct or indirect, of temperature, seems all but certain.

6. In addition to the rather remarkable constancy of the ratio of rates at constant temperature, we have also seen that the value of this ratio changes at different temperatures, but that this change is comparatively slight. Again we appeal to Woodruff for a parallel case ('11, p. 353): ''A study of the curve of the division rate at the two temperatures shows that temperature, as is well known, markedly influences the rate, but it also shows that the rhythms persist—the reproductive activity being, as it were, pitched at a higher scale, but its character is in no wise altered.'' Perhaps, in our case the changed value of the ratio of rates is the quantitative expression of 'change of pitch.'

7. Lastly, a word might be said about the long-period rhythm. It has been suggested that cycles in the life periods of protozoa have a definite correlation with conjugation. In this respect, therefore, the long-period rhythms in the present investigation have no relation whatever to Woodruff's cycles. But in another sense, perhaps, there is some analogy. For in the case of the locomotor rate rhythm, too, there is a periodicity not merely of the physiological processes, but also of the successive maxima of these processes (fig. 8). Again, in another respect, the analogy proves to be correct. Woodruff has found that when the environment of his cultures is changed, "cycles do NOT occur, but rhythms persist" ('11, p. 357). By reason of this statement and other similar ones, some doubt has been cast upon the existence of 'cycles' in the reproductive activity of Paramecium. In the present investigation, however, it can be shown from a comparison of the performance graphs that while short-period and long-period rhythms are clearly evident in uniform temperatures, the long-period rhythm tends to disappear—or perhaps it is obscured—when the temperature is changed, presumably until the organism has been adapted to the new environment. Among all the performance records there is, unfortunately, not a clear case from which the persistence or non-persistence of the long-period rhythm could be clearly demonstrated, for such a demonstration would demand observation of the same amoeba at two different, sufficiently diverse, temperatures—a demand that is not easy of fulfilment, in view of the experimental difficulties that are involved.

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THE INFLUENCE OF TEMPERATURE ON THE RATE OF LOCOMOTION IN AMOEBA

II. THE RATE OF LOCOMOTION IN AMOEBA AT DIFFERENT TEMPERATURES[1]

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AUTHOR'S ABSTRACT

In general, the changes of locomotor rate of Amoeba under the influence of temperature are similar to the effects of temperature in other biological processes.

The rate increases with rising and decreases with falling temperatures within a certain temperature range, this being, for the forms investigated, the interval between 6° and 23.5°C. In some individuals, however, these limits were found to be wider, extending from 2° to 26°. There is an average optimum at about 23.5°, beyond which an increase in temperature is followed by a decrease in rate. The immediate response of Amoeba to a change of temperature is variable, due, as was shown in a previous paper, to the fact that locomotor-rate changes are rhythmic. The effect of a change of temperature is thus conditioned not only by the environmental factors, but also by the phases of the locomotor rhythm. This fact also makes it difficult to establish the exact quantitative relationship which exists between a change of temperature and the locomotor rate—an aspect which will be developed in a future paper.

In a previous paper (Schwitalla, '24), to which the reader is referred for details regarding materials and methods, it was pointed out that the rate of locomotion in Amoeba is rhythmic and is, therefore, variable even when the temperature conditions are constant. This complicates the problem under consideration, for it thus becomes impossible to discover the relations between temperature and locomotion by studying the latter merely at a given cross-section of time.

Temperature may vary in intensity and in rate of change, and these variations may be frequent or infrequent. It is

[1] Contributions from the Zoölogical Laboratory of the Johns Hopkins University. This paper, together with one that preceded and one that is to follow, was submitted in 1921 in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Johns Hopkins University. The problem was suggested by Prof. S. O. Mast and the work was carried on under his direction. The author hereby acknowledges his great obligations to Professor Mast, to Prof. H. S. Jennings, and to his coworkers in the department.

highly probable that each of these modes of variation affects differently the rate of locomotion. Moreover, a change defined as a certain number of degrees determines, necessarily, neither the intensity of stimulation nor the magnitude of the response. A change of the merest fraction of a degree near the maximal or minimal limits of tolerance may be a much more effective stimulus than a change of several degrees near the optimal region, while the temperature susceptibility and the acclimatizing power, both variable and dependent upon other physiological conditions as well as on temperature, cannot be neglected in interpreting a given reaction. With these complications in mind, there is need, obviously, of the utmost caution in delimiting the application of conclusions in this subject.

THE IMMEDIATE RESPONSE IN RATE OF LOCOMOTION TO A CHANGE OF TEMPERATURE

The immediate response of Amoeba to a change of temperature is by no means uniform. This variability is illustrated by the typical instances cited in table 1. For the sake of clearness, the table is divided into two parts, the response to falling being separated from that to rising temperature, and in each of these parts the response to slight changes is separated from that to larger ones. Arbitrarily, we have considered a change of 8° or less as a slight change.

Individuals XV and XXIII illustrate the most common form of response—a decrease in rate when the temperature is lowered. The magnitude of the retardation, however, is decidedly different in the two cases and seems to bear no relation to the temperature change. Diversities from this mode of response are frequent. Thus individual XXXVIII responded to a decrease in temperature from 21° to 9.5° by an acceleration and a subsequent retardation; individual XV responded to a smaller change in temperature by a retardation and a subsequent acceleration. In the case of individual XL, a fall of only 1° was followed by a decided increase in rate, while a fall of 1.5° near the same temperature level was

followed by a maintenance of the old rate in the case of individual LIII.

A rise in temperature may occasion no less diversity in response. Generally, a rise in temperature is accompanied by an acceleration in rate, as occurred, for example, in the case of individuals I, LIII, and XXXIII. But that this is not

TABLE 1

The immediate locomotor-rate response of Amoeba to a change in temperature

	INDIVIDUAL	PREVIOUS TEMPERATURE °C.	RATE AT PREVIOUS TEMPERATURE[1] MM. PER MIN.	NEW TEMPERATURE °C.	RATES AT NEW TEMPERATURE[1] MM. PER MIN.	
					Immediate	Subsequent
			Falling temperature			
For larger fall	XXII	25	14	6	12	Rest
	XXXII	14	4.3	6	2	1.3
	XVI	28	2.3	11	2 (5 min.)	Rest
	XV	18.5	3.75	9.5	1.75	4
	XXXVIII	21	6	9.5	7	3
For slight fall	XL	21	3	20	5	9
	LIII	25.5	2	24	2	5
	XXX	27	9	25	3	9
	XXIII	28	15	25	7	6, 1.5
	XXIV	23	11	20	8	5, 11
	XVI	30	2	28	6.5	4, 8
			Rising temperature			
For larger rise	I	9	1.75	24	3.5	18
	I	10	1.7	24	5	6.5, 10
	LVI	16.5	9.3	29	3	5.5
	LIII	17.5	8	25	15.5	4.25
	LIV	17	9	25.5	8	19.5
For slight rise	XXXIII	20	3	25	4	8, 10
	XXXIII	25	6	28	10	11
	XLVI	10	3.3	14	3.3	2.2
	XLVI	14	3.1	18	2.7	
	XXVI	24	9.5	26.5	5	8, 9.2
	XVI	26	8	36	5.3	8

[1] The rates are given in terms of the magnification of the optical system, and hence are apparent. To find true rate, divide values here given by 64.

necessarily the case is shown, for example, by individual LVI for a large increase in temperature and by individuals XXVI and XLVI for smaller increases. In the supra-optimal range, a fall in temperature toward the optimum may occasion an acceleration, while a rise farther away from the optimum may occasion a retardation, as occurred, for example, in the case of individual XVI, but even this response is by no means uniform.

Instances of such diversities in response might readily be multiplied. From them it becomes clear that when the temperature is lowered through a small interval, especially when the change occurs near the physiological optimum, the rate of Amoeba may be unaffected. When the fall in temperature is greater, however, the rate may be retarded suddenly or gradually, or, in rare cases, there may be even an acceleration for a brief time. There is some indication that the temperature limits within which the lowering takes place has considerable effect upon the character of the response, the greatest change in the rate being expected when the drop occurs from optimal to minimal conditions. When the temperature is raised, there is usually an increase in rate, but sometimes there is a retardation. In none of these cases, however, is there evident in our data any strict proportionality between the intensity of the change in temperature and the magnitude of the change of rate. A slight fall in temperature of only 3°, and that, too, from supra-optimal toward optimal conditions, reduced the rate from 15 to 7 mm. per minute (individual XXIII), while, conversely, a large drop from 28° to 11° reduced the rate from 2.3 to 2 mm. per minute (individual XVI). A slight rise from 25° to 28°, and, therefore, away from the optimum, increased the rate in one case (individual XXXIII) from 6 to 10 mm. per minute, while a considerable rise in another case (individual LIV) was accompanied by a retardation in rate.

These quantitative anomalies suggest the explanation that has already been touched upon in our previous paper. It seems highly probable, on the basis of our data, that the

immediate response of Amoeba to a change of temperature is conditioned not only by the temperature itself, by the extent of the change, and by the position along the temperature range where the change takes place, but also by the particular phase of both the long-period and the short-period rhythms in which the organism happens to be at the instant when the environmental condition is altered. The response of the animal will be different if the temperature is raised, for example, while the rate is being accelerated, than if this is done while the rate is being retarded. Again, if the amoeba happens to be in the upward phase of the long-period rhythm, it is highly probable that an increase in temperature would produce a markedly greater increase in rate than if the increase occurred during the downward phase. The conclusion is all but forced upon one who examines the data at hand that the primary effect of temperature is to affect the rhythm, and only conditioned upon this the rate of locomotion. For a parallel case we might appeal to Woodruff, as we have done in another place ('24), bearing in mind, to be sure, the provisos there laid down: Woodruff and Baitsell say ('11, p. 149) "Records of a line of cells with the descending phase of the rhythm predominant, subjected to 28 degrees, and a line of cells with the ascending phase predominant, subjected to 24.5 degrees, may actually show (during the persistence of the rhythms) a more rapid rate of division at the lower than at the higher temperature. Accordingly, in this study it has been necessary to be sure that the animals were in comparable phases of the rhythm, or that the experiments were sufficiently prolonged to include one or more complete rhythms. It is clear that rhythms are a factor which must be taken into consideration in any study of the physiology of this animal." It would have been highly desirable that in the present study, too, all the amoebae had been in "comparable phases of their rhythm," but this was manifestly impossible of achievement in view of the fact that the rhythm cannot be detected until all the readings that have been taken have been carefully examined.

One further case of a disproportion between the temperature change and the change in rate should be mentioned here, but it will be treated at greater length in the discussion of the temperature coefficient. When the temperature conditions are such that Amoeba is just able to move, this temperature being for most individuals between 6° and 10°, a slight rise in temperature, a whole or half a degree may occasion a disproportionately great response. The phenomenon is probably an illustration of a physiological 'critical point.'

THE LOCOMOTOR RATE IN DIFFERENT MAINTAINED TEMPERATURES

Despite the constantly varying rates of locomotion which are the expression of the rhythm in Amoeba, the average rates in maintained temperatures show considerable conformity with known facts regarding the influence of temperature on physiological processes. Since, however, the immediate response to a change of temperature is so very irregular, it becomes important in a study like the present to continue observations for a considerable time, until the effect of changing the temperature is dissipated and the acclimatizing power of the organism becomes assertive. Thus only can a true average rate be found. In the course of this investigation, many of the amoebae were studied at constant temperatures for prolonged time intervals, some for more than three hours. Of the 366 temperature changes to which the sixty-two amoebae tested were subjected, 47 per cent were maintained for more than fifteen, and 36 per cent for more than twenty minutes. The average rate of locomotion was found by dividing the total distance traversed by the number of minutes during which the organism was in a state of locomotion. It was sometimes possible in this way to observe the same individual for comparable time intervals in several different temperatures. The data given in table 2 were selected from the performance records of the various individuals. The instances are practically random selections.

A. Comparative average rates of locomotion of the same individual at different maintained temperatures

Much of the data in this table substantiates the general fact that the average rate up to optimal conditions increases with rising and decreases with falling temperature. As examples we may cite, for rising temperature, individuals XXI and LII, and for falling temperatures, individuals XX and XXVII. The same regularity may be observed in some cases even when there were frequent, short-period alternations of high and low temperatures, as may be seen from the record of individual XXXV. On the other hand, there are numerous increases in rate with falling, just as there are decreases in rate with rising temperature. From this latter class of cases there must, of course, be excluded those observations which took place at temperatures higher than the optimum, as happened, for example, in the case of individual XXVIIIa. The record for individual XXVIII furnishes a fair example of the diversities of behavior. As the temperature rose from 21° to 23° and then to 24°, the average rate varied in the same sense. A further rise in temperature to 27°—beyond the optimum, as will be seen below—effected a decrease in average rate. Then there followed a rise in temperature to 28°, which occasioned a further increase in average rate, although a decrease was expected, and such a retardation did, in fact, take place when the temperature rose further to 30° and then to 32°. At the final temperature of 23°, the animal was moving at a rate lower than its rate at 21° at the beginning of the experiment.

In this instance there was a reduction in average rate when the temperature rose into the supra-optimal region. Such a retardation in the average rate, however, may occur also when the increase is on the suboptimal side of the temperature range. Thus, in the case of individual XXX, the rate was decreased by one-third when the temperature rose from 20° to 25°. Again, in the case we are discussing, it might well be that the intervals during which the animal was under

TABLE 2

Average rates of locomotion of certain selected amoebae at different temperatures

INDIVIDUAL	TEMPERATURE, °C.	TOTAL DISTANCE, MM.	DURATION OF OBSERVATION, MINUTES	RATE[1], MM. PER MINUTE
III	22	71.0	8	8.87
	10	63.25	39	1.62
	25	44.5	4	11.10
	11	79.5	28	2.84
	25	59.0	7	8.43
	11	28.5	26	1.10
VI	20	85.5	8	10.69
	9.5	31.0	16	1.94
	24	99.0	13	7.62
	10	35.0	16	2.20
	24	89.5	8	11.20
	10	11.5	20	.58
	20	51.5	8	6.44
	10	28.0	12	2.33
	24	22.5	5	4.50
XVI	20	100.5	46	2.18
	26	41.0	10	4.10
	27	17.5	3	5.80
	26	15.0	2	7.50
	24	42.0	7	6.00
	26	9.0	2	3.50
	28	12.0	2.5	4.80
	26	44.0	5	8.80
XVIII	20	179.5	67.5	2.66
	16	370.0	115	3.22
	20	100.0	28.5	3.50
XX	20	129.0	51.5	2.50
	11	64.0	45	1.42
XXI	22.5	271.5	48.5	5.59
	26	371.5	56	5.67

[1] The rates are given in terms of the magnification of the optical system, and hence are apparent. To find true rate, divide values here given by 64.

observation were too brief to find its true average rate. Individual XXX, however, was observed for twenty-seven minutes both at 20° and at 25°, and still the average rate at 25° was lower than at 20°. 'Fatigue' phenomena, which are suggested by the low average rate of individual XXVIII at 23°

TABLE 2—*Continued*

INDIVIDUAL	TEMPERATURE, °C.	TOTAL DISTANCE, MM.	DURATION OF OBSERVATION, MINUTES	RATE, MM. PER MINUTE
XXVII	21	204.0	29	7.03
	15	69.0	13	5.30
XXVIII	21	113.5	27.5	4.13
	23	28.5	6.75	4.22
	24	123.0	23	5.30
	27	30.5	6.66	4.60
	28	37.5	6	6.25
	30	29.0	5.5	5.30
	32	22.0	5	4.40
	23	6.0	2	3.00
XXVIIIa	24	30.0	3.65	8.33
	28	41.0	6	6.83
XXX	20	171.0	27	6.33
	25	122.0	27	4.52
	27.5	95.0	13	7.30
	22	160.5	15	10.70
	20	98.5	16	6.16
	16	12.0	2	6.00
	15	144.5	29	4.98
	27	30.0	12	2.50
XXXV	19.5	134.5	24.96	5.39
	11	5.0	6	.83
	12	9.5	3	3.20
	13	10.0	4	2.50
	14	19.0	8	2.38
	15	73.0	22	3.32
	12	23.0	10	2.30
LII	18	181.5	27	6.72
	22	129.0	15.5	8.32

after it had been moving for eighty consecutive minutes, are noticeably lacking in our data. Thus, individual XVIII moved at a greater rate at a final temperature of 20° during 28.5 minutes, after it had been moving for 115 minutes at 16°, than it did in 20° for 67.5 minutes in the early part of the experiment. Some of the anomalies in behavior may readily be harmonized, however, if different individuals have different optima, and there are a considerable number of indica-

tions throughout our data that this feature should be emphasized.

We might analyze in the same way the phenomenon that occurs frequently enough—an increase in average rate when the temperature rises. It may suffice, however, to refer the reader to the record of individual XVI as typical. Again, it would be instructive to compare the rate of one individual when it is repeatedly subjected to the same temperature with various intervening environmental temperature changes. Individual III furnishes an instance, as it was twice subjected to a temperature of 10° for twenty-eight and twenty-six minutes, after it had been exposed for brief periods to sudden changes in high temperature. In the case of individual VI, we may compare three average rates at 24°, two at 20°, and three at 10°, all for comparatively brief periods of time. A further analysis, however, would but corroborate the statements that have already been made.

Thus far we have dealt chiefly with the qualitative aspects of the change of rate, speaking of it merely in terms of acceleration and retardation, or of increase and decrease. A study of the quantitative relations would lead practically to the same conclusions, although in this way the lack of proportion in the temperature as compared with the magnitude of the change of rate would be still more emphasized.

We may now summarize this brief discussion of the data presented in table 2. When the same individual is subjected for prolonged time intervals to various maintained temperatures within the suboptimal range, the average rate usually increases with rising and decreases with falling temperature; within the supra-optimal range, however, the average rate decreases with rising and increases with falling temperature. At times, however, both within the suboptimal and the supra-optimal ranges, the average rate varies in a manner contrary to this expectation. In the present investigation, out of 316 temperature changes, approximately 60 per cent of the averages conform to what might be termed 'the temperature law,' while 40 per cent of the cases did not

conform. As far as is discoverable from our data, neither the level from which the change takes place, nor the duration of locomotion, nor the magnitude of the temperature change need necessarily determine quantitatively the kind of change of average rate that will ensue.

B. Comparative average rates of locomotion at different maintained temperatures of all the amoebae studied

It would serve no useful purpose to publish in tabular form the average rates of all the amoebae at the various temperatures at which they were studied. To make the general trend of the results of this investigation clear, however, all these averages have been plotted in figure 1. The abscissae represent temperatures, the ordinates average rates of locomotion. The average rate, multiplied by 64, of each individual at a particular temperature is represented by a small circle.

The outstanding features of this figure may be briefly indicated. The great variability of the average rates is very apparent. Thus at 10° the average rates varied between 0.5 and 4.45 mm. per minute; at 20°, between 1.2 and 10.7 mm. per minute; at 30°, between 5.3 and 6.5 mm. per minute. Just as striking, however, is the gradual upward trend of the average rates from 6° to a region between 22° and 25° and then its downward trend beyond that temperature. Obviously, we are here dealing with a process that conforms to other physiological processes. There is an increase in the rate of the process to an optimum and then a decrease. A curve of average rates is plotted in the figure. It was calculated by adding the total distance traversed by all the amoebae studied at a given temperature and dividing this sum by the sum of the total number of minutes during which all these individuals were in active locomotion. This curve, therefore, represents the average rates of all individuals studied at a particular temperature. Obviously, the curve is far from being smooth, but its general tendency is clear. The abrupt changes in the curve at 12°, 15°, 20°, 22°, and 25° may be

significant, but thus far no meaning can be attached to them. It will be noted that two maxima are indicated, although the general character of the curve would lead one to expect a plateau between 22° and 25°. In an effort to find this plateau, the averages for 22° and 23° and for 24° and 25° have been combined, and the resultant values are shown by the dotted line between 21° and 26°.

To smooth out this curve of averages, the usual statistical expedient was adopted of grouping the findings around salient temperatures. Hence, if we consider as the 10° group all the rates at temperatures between 7° and 12.5°, as the 15° group all those between 13° and 17.5°, and so on, we may plot a 5°-interval curve at the middle points of those various ranges. This curve, too, has been inserted in the figure with its appropriate designation.

Finally, it has already been intimated that despite the great variation in the rate of locomotion at any particular temperature, it is possible that temperature may fix a maximum beyond which the rate cannot be accelerated at that particular temperature. This would stress the view that, even though the rate of locomotion is dependent upon whatever factors influence the variations and the rhythm, Amoeba must still move in accordance with the physical and the chemical properties, fluidity and elasticity, of its colloidal system. It is interesting to examine this statement with the combined results of this investigation before us. A curve of maximal average rates has accordingly been inserted in the figure. It will be noted that the maximal rate increases from 6° to 21.5°, and then begins to decline with but two average rates, one at 25.5°, the other at 27°, to interfere with the smooth descent. The meaning which might well be taken from this curve is perhaps this, that, say at 17° for example, Amoeba may move at any rate below an apparent rate of 8.6 mm. per minute, the point at which the ordinate for 17° crosses the curve of maxima. This corresponds to a rate of 13.4 μ per minute. For 21° this point would be at 11.9 mm. per minute, corresponding to a rate of 18.5 μ per minute. The maximum of

the curve of maxima, at 21.5° would seem to lie close enough to the maximum of the curve of rates not to invalidate this interpretation.

A glance at the figure will show an apparent lack of uniformity in the distribution of the data along the temperature scale. There are fewer average rates for extreme temperatures than for those near the middle of the range, and many more for whole degrees than for half degrees. The former fact need not be as disturbing as it may seem at first sight, for if larger temperature intervals, say of 4° are considered, the observations are fairly evenly distributed, except at the extremes. Justification for this may be found in the fact that at the lower temperatures observation becomes extremely difficult, while at the higher temperatures there is constant danger of injuring the animal and of thus losing an otherwise successful series of observations. As for the relative lack of data for the half-degree intervals, a consistent effort was made to insure facility in the observations by securing whole-degree temperatures. It is felt that more perfect distribution of the data would change the general results by little more than a fraction of a millimeter in rate or a fraction of a degree in temperature.

SUMMARY

1. The immediate response of Amoeba to both rising and falling temperatures is variable, both qualitatively and quantitatively. This is probably due to the fact that different individuals at the time when they were studied were not in 'comparable phases of their rhythm.'

2. When average rates at different temperatures for the same as well as for different individuals are compared, however, there is in general an increase of rate with rising and a decrease of rate with falling temperature within the suboptimal temperature range, that is up to approximately 25°. At higher temperatures there is usually a decrease in rate with rising and an increase in rate with falling temperature.

3. The maximal average rate also increases progressively from 6° to approximately 21.5°, paralleling roughly the increase in average rates, and then decreases abruptly. The maximal rates may be taken as limiting values of the rates attainable by Amoeba at a particular temperature.

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BIOGRAPHICAL

Alphonse M. Schwitalla was born in Upper Silesia, Germany, in 1882, and came to this country in 1885. He graduated from High School in 1899 in St. Louis, Missouri. After entering the Jesuit Order, he received the A. B. degree in the College of Arts, St. Louis University, St. Louis, Missouri, in 1906. The Master's Degree was conferred on him in 1907 in the Philosophical Department of the same University. From 1907 to 1910 he acted as Instructor in Chemistry at St. Xavier's College, Cincinnati, Ohio, and then continued his studies in Biology, while acting as assistant in the Department of Physiology, at the Medical School of St. Louis University during the two ensuing years. He entered the Theological Department of the same University in 1912, was ordained priest in 1915, and finished his theological course in 1916. The years 1917-1919 he spent as Instructor in Chemistry and Biology at Rockhurst College, Kansas City, Missouri. He entered Johns Hopkins University in 1919. His principal subject was Zoology, his subordinate subjects Plant Physiology and Physical Chemistry.